Natl. Acad. Sci. Lett. (July–August 2013) 36(4):393–395
DOI 10.1007/s40009-013-0145-z

RESEARCH ARTICLE

The Interaction of Ferric Ions with Jack Bean Urease by Isothermal Titration Calorimetry

G. Rezaei Behbehani · L. Barzegar

Received: 8 July 2012 / Revised: 8 February 2013 / Accepted: 1 April 2013 / Published online: 7 August 2013

Abstract The aim of the investigations was to measure the influence of Fe^{3+} ion on jack bean urease (JBU) activity. Interaction between Fe^{3+} and JBU, is examined using isothermal titration calorimetry. It was found that Fe^{3+} ions acted as a non-cooperative inhibitor of JBU, and there is a set of 12 identical and independent binding sites for Fe^{3+} ions. The small structural parameters show that there are little changes on the JBU structure, indicating that Fe^{3+} has minor effect on the JBU activity. The association equilibrium constant is 42,484.13 ± 110 mol l^{-1}, indicating the moderate interaction of Fe^{3+} ion with JBU. The molar enthalpy of binding is $\Delta H = -4.33$ kJ mol^{-1}.

Keywords Isothermal titration calorimetry · Jack bean urease · Fe^{3+} ion · Binding parameters

Introduction

There are a variety of nitrogenous fertilizers available in the market; however, urea consumption is 38 %, which is higher than other nitrogenous fertilizers due to the relatively low manufacturing cost and high concentration of N [1]. Jack bean urease (JBU) rapidly catalyzes the hydrolysis of urea to form ammonia and carbon dioxide. The product, ammonia, of such decomposition reactions diffuses across the cytoplasmic membrane, buffering the periplasmic space and allows growth in the presence of extracellular gastric acid and responsible for negative effects of urease activity in human health, such as causing peptic ulcers and stomach cancer. Besides, in agriculture the efficiency of soil nitrogen fertilization with urea decreases due to ammonia volatilization and root damage caused by soil pH increase [2–4].

Therefore, it is interesting to control the activity of urease through the use of its inhibitors in order to counteract these negative effects in medicine, environmental and agronomic. Heavy metal ions inhibit both plant and bacterial urease at the following approximate order of effectiveness: $Hg^{2+} \approx Ag^{+} > Cu^{2+} > Ni^{2+} > Cd^{2+} > Zn^{2+} > Co^{2+} > Fe^{3+} > Pb^{2+} > Mn^{2+}$ with Hg^{2+}, Ag^{+} and Cu^{2+} ions practically known as the strongest inhibitors [3–6]. The objective of this study was to assess the urease activity and conformational changes of JBU due to its binding to Fe^{3+} ion.

Materials and Methods

Jack bean urease (JBU; MW = 545.34 kDa), Tris salt and Fe^{3+} ions obtained from sigma chemical Co. The isothermal titration microcalorimetric experiments were performed with the four channel commercial microcalorimetric system. Fe^{3+} solution (4 mmol l^{-1}) was injected by use of a Hamilton syringe into the calorimetric titration vessel, which contained 1.8 ml JBU (37 μmol l^{-1}). Injection of Fe^{3+} solution into the perfusion vessel was repeated 28 times, with 10 μl per injection. The calorimetric signal was measured by a digital voltmeter that was part of a computerized recording system. The heat of each injection was calculated by the ''Thermometric Digitam 3'' software program. The heat of dilution of the Fe^{3+} solution was measured as described above except JBU was excluded. The microcalorimeter was frequently calibrated electrically during the course of the study.

G. R. Behbehani (✉) · L. Barzegar
Chemistry Department, Faculty of Science, Islamic Azad University, Takestan Branch, Takestan, Iran
e-mail: grb402003@yahoo.com

Table 1 The heats of Fe^{3+}+JBU interaction at 300 K in 30 mmol l^{-1} Tris buffer solution of pH = 7

$[Fe^{3+}]$ (μmol^{-1})	q (μJ)	q_{dilut} (μJ)
33.06	−6,120.00	944.91
54.79	−5,850.82	1,597.11
76.29	−5,634.384	2,205.39
97.56	−5,477.38	2,786.80
118.60	−5,131.78	3,342.35
139.41	−4,996.38	3,873.63
160.00	−4,860.49	4,473.49
180.37	−4,721.70	4,980.63
200.53	−4,471.57	5,449.09
220.47	−4,350.68	5,899.80
240.21	−4,212.91	6,336.38
259.74	−4,050.98	6,760.73
279.07	−3,865.98	7,164.13
298.20	−3,938.68	7,561.13
317.13	−3,798.76	7,944.76
335.88	−3,722.18	8,315.17
354.43	−3,585.40	8,673.31
372.80	−3,439.11	9,013.68
390.98	−3,365.55	9,352.72
408.98	−3,305.17	9,680.65
426.80	−3,237.45	9,997.58
444.44	−3,174.28	10,306.38
461.92	−3,103.56	10,605.02
479.22	−3,025.41	1,089.37
496.35	−2,970.58	11,179.26
513.32	−2,922.99	11,451.94
530.32	−2,863.82	11,717.48
546.76	−2,816.68	11,974.28

The precisions are 0.1 nJ or better

Results and Discussion

The obtain results were reported in Table 1 and shown graphically in Fig. 1.

We have shown previously that the heats of the ligand + JBU interactions in the aqueous solvent mixtures can be calculated via the following equation [7–12]:

$$q = q_{\max} x'_B - \delta^{\theta}_A (x'_A L_A + x'_B L_B) - (\delta^{\theta}_B - \delta^{\theta}_A)(x'_A L_A + x'_B L_B) x'_B \quad (1)$$

q is the heat of Fe^{3+} + JBU interaction and the optimized value of $q_{\max}$ represents the heat value upon occupation of all binding sites on JBU. The parameters δ^{θ}_A and δ^{θ}_B are the indexes of JBU stability in the low and high Fe^{3+} concentrations, respectively. If the binding of a ligand at one site increases the affinity for that ligand at another site, then the macromolecule exhibits positive cooperativity. Conversely, if the binding of a ligand at one site lowers the affinity for that ligand at another site, then the enzyme exhibits negative cooperativity. If the ligand binds at each site independently, the binding is non-cooperative. x'_B can be expressed as follows:

$$x'_B = \frac{p x_B}{x_A + p x_B} \quad (2)$$

One can express x_B fractions, as the Fe^{3+} concentrations divided by the maximum concentration of the Fe^{3+} upon saturation of all JBU as follows:

$$x_B = \frac{[Fe^{3+}]}{[Fe^{3+}]_{\max}}, \quad x_A = 1 - x_B \quad (3)$$

$[Fe^{3+}]$ is the concentration of Fe^{3+} and $[Fe^{3+}]_{\max}$ is the maximum concentration of the Fe^{3+} upon saturation of all JBU. L_A and L_B are the relative contributions due to the fractions of unbound and bound metal ions in the heats of dilution in the absence of JBU and can be calculated from the heats of dilution of Fe^{3+} in the buffer solution, q_{dilut}, as follows:

$$L_A = q_{dilut} + x_B \left(\frac{\partial q_{dilut}}{\partial x_B}\right), L_B = q_{dilut} + x_A \left(\frac{\partial q_{dilut}}{\partial x_B}\right) \quad (4)$$

The heats of Fe^{3+}+JBU interactions, q, were fitted to Eq. 1 across the whole Fe^{3+} compositions. In the fitting procedure, p was changed until the best agreement between the experimental and calculated data was approached (Fig. 1). δ^{θ}_A and δ^{θ}_B values are recovered from the coefficients of the second and third terms of Eq. 1. The small relative standard coefficient errors and the high r^2 values (0.99999) support the extended solvation model. The binding parameters for Fe^{3+}+JBU interactions recovered from Eq. 1 were listed in Table 2. $P > 1$ or $P < 1$ indicate positive or negative cooperativity of a macromolecule for binding with a ligand, respectively; $P = 1$ indicates that the binding is non-cooperative.

For a non-cooperative interaction:

$$\frac{q_{\max} - q}{q_{\max}}[\text{JBU}] = \left(\frac{q_{\max} - q}{q}\right)[Fe^{3+}]\frac{1}{g} - \frac{K_d}{g}. \quad (5)$$

[JBU] and $[Fe^{3+}]$ are concentrations of JBU and Fe^{3+}, respectively. q represents the heat value at a certain Fe^{3+} ion concentration and the optimized values for q_{max} represents the heat value upon saturation of all JBU. Checking different values for $q_{\max}$, the best linear plot of $(\frac{q_{\max}-q}{q_{\max}})[\text{JBU}]$ versus $(\frac{q_{\max}-q}{q})[Fe^{3+}]$was approached as follows:

$$\frac{-3465 - q}{-3465}[JBU] = \left(\frac{-3465 - q}{q}\right)[Fe^{3+}]\frac{1}{12} - \frac{1.95}{12}. \quad (6)$$

Comparing Eqs. 5 and 6, the number of binding sites on JBU ($g = 12$) and the dissociation equilibrium con-

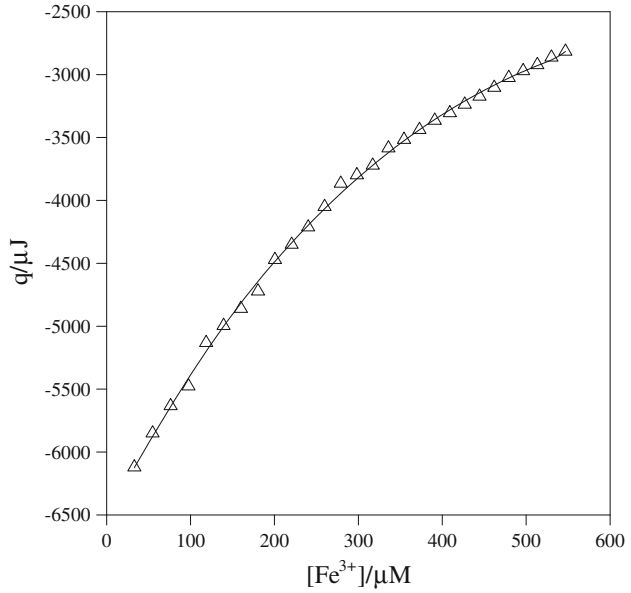

Fig. 1 Comparison between the experimental heats (*filled triangle*) at 300 K, for Fe^{3+} + JBU interactions and the calculated data (*lines*) via Eq. 1. The $[Fe^{3+}]/\mu M$ are the concentrations of $[Fe(NO_3)_3]$ solution in μmol l^{-1}

Table 2 Binding parameters for JBU + Fe^{3+} interactions

Parameters	T = 300 K
P	1 ± 0.01
δ_A^θ	−0.22 ± 0.04
δ_B^θ	0.004 ± 0.001
K_a / l mol^{-1}	42484.13 ± 110
ΔH / kJ mol^{-1}	−4.33 ± 0.09
ΔG / kJ mol^{-1}	−26.58 ± 0.09
ΔS/kJ $mol^{-1}K^{-1}$	0.07 ± 0.01

$P = 1$ indicates that the binding is non-cooperative. The small δ_A^θ and δ_B^θ values show that there are little changes on the JBU structure, indicating that Fe^{3+} has minor effect on the JBU activity. The association equilibrium constant indicates that JBU has moderate affinity to bind with Fe^{3+}

stant ($K_d = 1.95$ μmol l^{-1}) can be calculated. Dividing the optimized q_{max} amount of −3465 μJ (equal to −52.32 kJ mol^{-1}) by $g = 12$, gives $\Delta H = -4.34 \pm 0.09$ kJ mol^{-1}.

The change in standard Gibbs free energy ($\Delta G°$) can be calculated according to the equation (7), which its value can use in equation (8) for calculating the change in standard entropy ($\Delta S°$) of binding process.

$$\Delta G = -RT \ln K_a \quad (7)$$

$$\Delta G = \Delta H - T\Delta S \quad (8)$$

where K_a is the association binding constant ($K_a = 1/K_d$). The obtained value for K_a is 42,484.13 ± 110 L mol^{-1} Hence:

$\Delta G = -26.58 \pm 0.09$ kJ mol^{-1}

$\Delta S = 0.07 \pm 0.01$ kJ mol^{-1} K^{-1}

All thermodynamic parameters for the interaction between JBU and Fe^{3+} ion have been summarized in Table 2. The small and negative value of δ_A^θ indicates that Fe^{3+} is a poor inhibitor of JBU activity.

Acknowledgments The financial support of the Islamic Azad University of Takestan is gratefully acknowledged.

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